7 Effect of soil moisture on nodule population

Soil moisture plays a pivotal role in the growth and functioning of all plants and soil microorganisms with the growth response being proportional to the moisture level, up to a certain extent. Soil moisture levels also determine the availability of nutrients which are concentrated predominantly in the upper layers of the soil profile (Pinkerton & Simpson 1986). According to Bartels (1966) and Stiles (1966), the clover content in mixed swards is sensitive to soil moisture and could be increased by irrigation. However, Reid & Castle (1965) reported that irrigation had no significant effect on the proportion of clover. Data obtained from the field observations (Chapter 4) indicate that increased soil moisture prevailing during the rainy season may have beneficial effects on the growth of the clover.

Observations on the effects of soil moisture on the nodulated root system of legumes dates back to Wilson (1931) who first reported that drought adversely affected the root nodules of legumes. Later, Mansfield (1961) and Doku (1970) confirmed the beneficial effects of irrigation on nodule number and size reported earlier. Soil moisture conditions are known to affect (i) the survival and movement of rhizobia in soil (Hamdi 1971), (ii) the rate of infection of legume root hairs (Worrall & Roughley 1976), and (iii) nodule growth and function (Davey & Simpson 1989).

However, most of the work on this aspect has been confined to legumes like soybean which bear spherical, determinate nodules of limited growth and thus the observed effects of soil moisture are immediate on pre-formed nodules. T. repens on the contrary, bears elongated indeterminate nodules which are scattered all over the fibrous root system. Each nodule has an apical meristem which allows it to continue to grow and function over a considerable period of time.

The Shillong plateau is characterised by dry winter and spring whereas heavy rainfall is recorded during the rainy season with almost 85% of the total annual rainfall occurring during the months of June-September. Corresponding soil moisture levels also vary widely ranging from 8% in winter to 35% during the rainy season (Fig. 3.2). Thus the white clover populations are exposed to a wide range of soil moisture levels and, therefore, an analysis of the effects of soil moisture on the nodule population dynamics in the two leaf morph populations of the clover could be quite revealing.

MATERIALS AND METHODS

These have been described in Chapter 3

RESULTS

Plant growth

Figure 7.1a represents the variation in shoot dry weight with soil moisture levels in the two leaf morph populations of white clover. At nil soil moisture level, all the plants died within 12 days. The increasing soil moisture levels were associated with a significant ($P < 0.01$) corresponding increase in shoot weights in both the leaf morph populations. Though there was no significant difference in shoot weights between the two leaf morph populations, variations in shoot weights between the two harvests were highly significant (Table 7.1) with weights at H2 exhibiting a near three fold increase over those at H1.

Increasing soil moisture levels were associated with a corresponding significant ($P < 0.01$) increase in the root dry weights (Fig. 7.1b). At both harvests, the marked population exhibited a significantly ($P < 0.01$) greater root biomass. At H2, the biomass allocation pattern in the above- and below-ground portions are diametrically opposite in the two leaf morph populations; the marked population allocated significantly greater biomass to root, whereas the unmarked population allocated more biomass to shoot.

The total plant weights also showed a significant ($P < 0.01$)

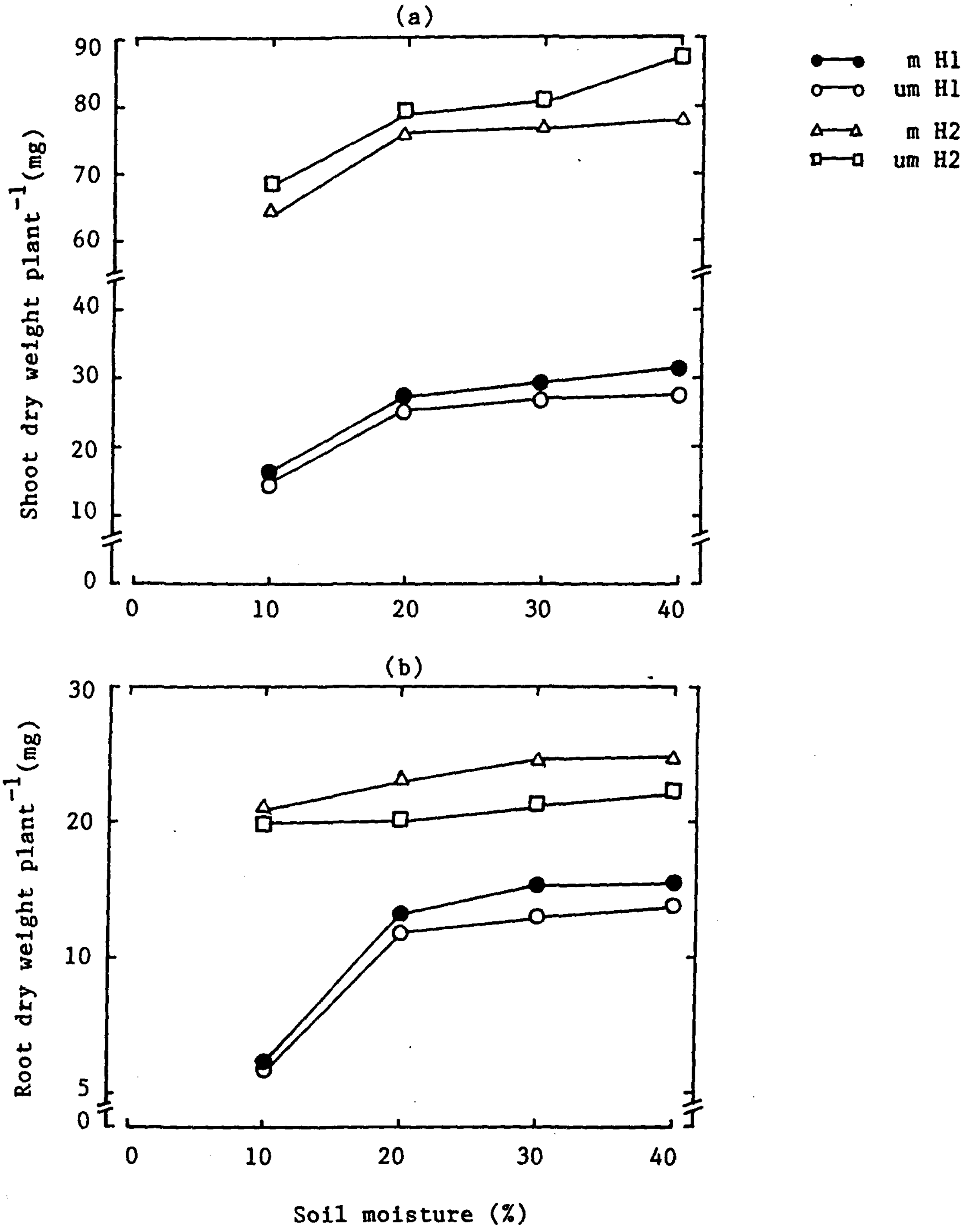

Fig.7.1. Effect of soil moisture on (a) shoot dry weight and (b) root dry weight per plant in the two leaf morph populations of the clover at the two harvests.

Table 7.1. Analysis of variance of the legume growth parameters as influenced by the harvests, leaf morph populations and different moisture regimes.

Plant parameters	Source of variation	df	F value	Level of significance
Shoot dry weight	Harvests	1	2985.46	P < 0.01
	Leaf morphs	1	3.92	ns
	Moisture %	3	52.80	P < 0.01
	Harv x Morph	1	11.83	P < 0.05
	Harv x Moist	3	0.49	ns
	Morph x Moist	3	0.30	ns
Root dry weight	Harvests	1	3760.38	P < 0.01
	Leaf morphs	1	86.47	P < 0.01
	Moisture %	3	223.49	P < 0.01
	Harv x Morph	1	8.19	ns
	Marv x Moist	3	63.61	P < 0:01
	Morph x Moist	3	3.77	ns
Plant dry weight	Harvests	1	2817.00	P < 0.01
	Leaf morphs	1	0.12	ns
	Moisture %	3	62.91	P < 0.01
	Harv x Morph	1	5.87	ns
	Harv x Moist	3	1.25	ns
	Morph x Moist	3	0.20	ns
PSA	Harvests	1	1571.10	P < 0.01
	Leaf morphs	1	5.00	ns
	Moisture %	3	316.56	P < 0.01
	Harv x Morph	1	0.50	ns
	Harv x Moist	3	64.50	P < 0.01
	Morph x Moist	3	0.33	ns

PSA = Photosynthetic area

ns = not significant

increase with increasing soil moisture levels. At H2 the dry weights showed a more than three fold increase ($P < 0.01$) although there was no significant difference in dry weights between the two leaf morph populations (Table 7.1).

Photosynthetic area

Increasing soil moisture levels were also associated with a significant ($P < 0.01$) increase in the PSA per plant and at H2 the values were more than twice that at H1. However, there was no significant difference in the PSA between the two leaf morph populations (Tables 7.1, 7.2).

Nodule number

Figure 7.2a shows the variation in mean nodule number per plant with increasing soil moisture content. At H1, the nodule number in both the leaf morph populations increased with increase in soil moisture up to 30% above which there was a conspicuous drop in the nodule number. At H2, the nodule number per plant increased only up to 20% soil moisture level above which the number dropped steeply. The marked population exhibited a sharp increase in nodule number over the unmarked population at H2 (Tables 7.3, 7.4).

Mean nodule weight

Though increasing soil moisture levels at H1 were associated with an increase in the mean weight per nodule, this trend was reversed at H2 (Table 7.3). There was no significant variation in the weight per nodule either amongst the moisture levels or between

Table 7.2. Effect of soil moisture on the nodule dry weight, plant N concentration and photosynthetic area (PSA) per plant in the two leaf morph populations of the clover at the two harvests.

Soil moisture (%)	Harvest 1						Harvest 2					
	Marked population			Unmarked population			Marked population			Unmarked population		
	Nodule dry weight (μg)	Plant N concentration (mg g^{-1})	PSA per plant (mm^2)	Nodule dry weight (μg)	Plant N concentration (mg g^{-1})	PSA per plant (mm^2)	Nodule dry weight (μg)	Plant N concentration (mg g^{-1})	PSA per plant (mm^2)	Nodule dry weight (μg)	Plant N concentration (mg g^{-1})	PSA per plant (mm^2)
10	37.6	20.13	131.92	36.9	18.60	130.54	70.3	40.80	904.00	72.0	35.90	928.00
20	43.3	23.60	320.96	40.3	24.00	339.09	61.0	38.20	1028.00	70.2	35.07	1092.67
30	45.0	32.11	628.16	43.2	27.30	701.96	40.2	33.04	1125.80	56.4	30.10	1143.12
40	48.1	33.07	973.00	47.0	27.61	965.21	37.9	29.18	1228.20	48.8	27.62	1289.92

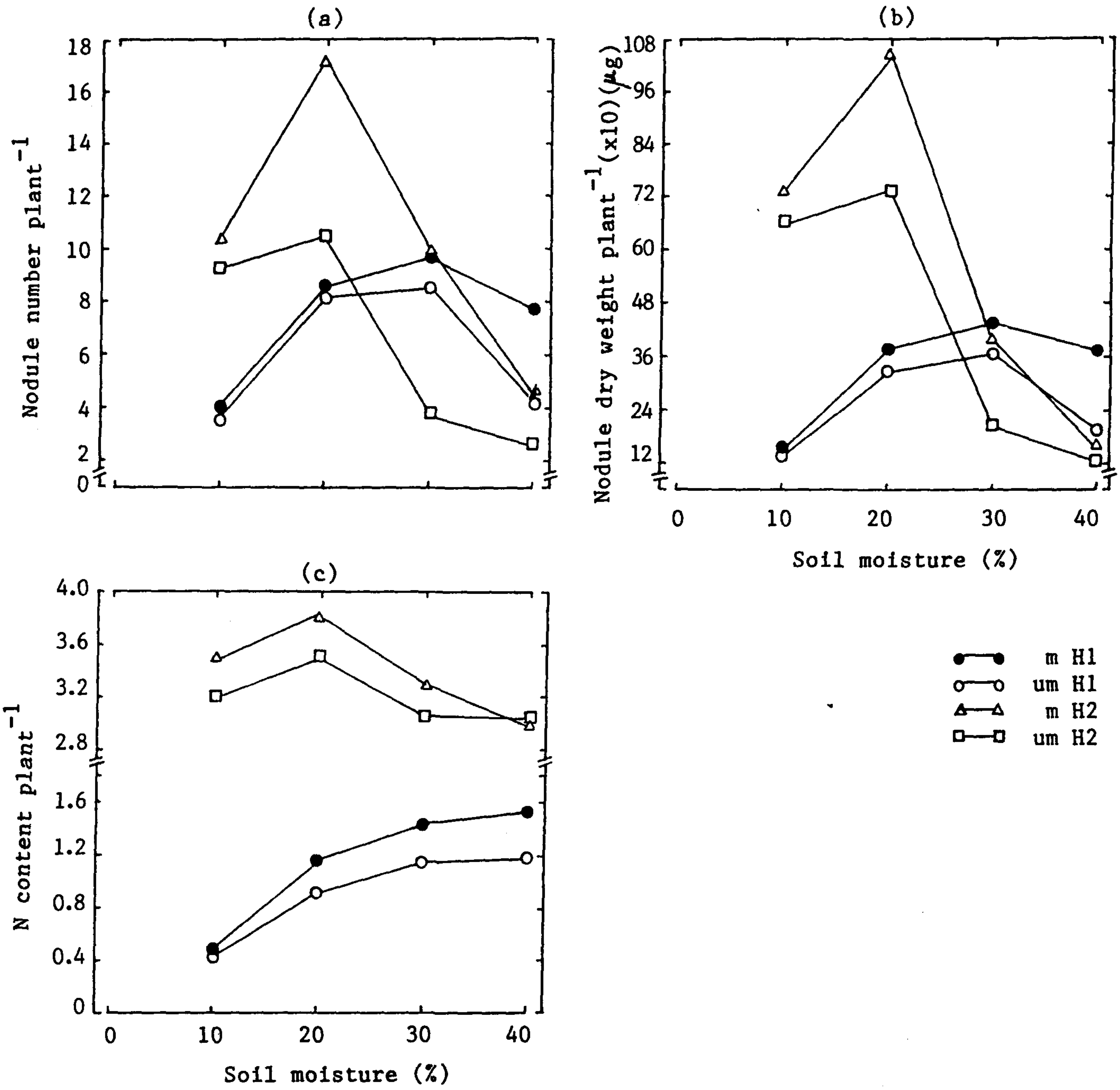

Fig.7.2. Effect of soil moisture on (a) nodule number (b) nodule dry weight and (c) N content per plant in the two leaf morph populations of the clover at the two harvests.

Table 7.3. Analysis of variance of nodule characteristics of the clover as influenced by the harvests, leaf morph populations and different moisture regimes.

Nodule characteristics	Source of variation	df	F value	Level of significance
Nodule number	Harvests	1	3.02	ns
	Leaf morphs	1	7.88	ns
	Moisture %	3	7.89	ns
	Harv. x Morph	1	1.98	ns
	Harv. x Moist	3	6.01	ns
	Morph x Moist	3	0.52	ns
Mean nodule weight	Harvest	1	79.74	$P < 0.01$
	Leaf morphs	1	5.90	ns
	Moisture %	3	8.48	ns
	Harv. x morph	1	11.91	$P < 0.05$
	Harv. x Moist	3	29.34	$P < 0.05$
	Moist x Morph	3	0.77	ns
Nodule weight per plant	Harvests	1	23.34	$P < 0.05$
	Leaf morphs	1	6.91	ns
	Moisture %	3	15.27	$P < 0.05$
	Harv. x Morph	1	0.87	ns
	Harv. x Moist	3	19.53	$P < 0.05$
	Moist x Morph	3	0.44	ns
Total N $plant^{-1}$	Harvests	1	951.60	$P < 0.01$
	Leaf morphs	1	9.12	ns
	Moisture %	3	7.41	ns
	Harv. x Morph	1	0.03	ns
	Harv. x Moist	3	16.27	$P < 0.05$
	Morph x Moist	3	0.44	ns

Table 7.4. Effect of soil moisture on the nodule number per unit (100 mg) plant dry weight.

Soil moisture (%)	Harvest 1		Harvest 2	
	marked population	unmarked population	marked population	unmarked population
10	17.14	16.98	12.08	10.34
20	21.03	21.01	17.14	10.32
30	21.36	19.91	9.64	3.52
40	16.31	9.60	4.28	2.36

the two leaf morph populations. However, at H2 the mean weight per nodule was significantly ($P < 0.01$) higher than that at H1 (Table 7.3).

Nodule weight per plant

The nodule weight per plant (Fig. 7.2b) showed a trend similar to that of nodule number per plant, and varied significantly ($P < 0.01$) between the soil moisture levels. Between the two leaf morph populations, there was no significant variation, although the marked population showed consistently higher values at both harvests. At H2, the nodule mass per plant had increased considerably and was significantly ($P < 0.01$) greater than that at H1.

Total N per plant

At H1, the total N content per plant showed a steady increment with increasing soil moisture levels in both the leaf morph populations. However at H2, the total N content increased up to 20% soil moisture after which the values decreased with a corresponding further increase in soil moisture levels. Although there was no significant variation in total N per plant either amongst the soil moisture levels or between the two leaf morph populations, values at H2 showed a three fold increase ($P < 0.01$) as compared to values at H1 (Table 7.3).

Relative growth rate and relative N accumulation rate

The relative growth rates (R_W) of the two leaf morph populations at varying soil moisture levels are given in Table 7.5. At H1, R_W

Table 7.5. Effect of soil moisture on relative growth rate (R_W)(μg d^{-1}) and relative N accumulation rate (R_N)(μg d^{-1}) in the two leaf morph populations of the clover at the two harvests.

Soil moisture (%)	Harvest 1				Harvest 2			
	Marked population		Unmarked population		Marked population		Unmarked population	
	R_W	R_N	R_W	R_N	R_W	R_N	R_W	R_N
10	7.27	10.78	10.12	15.60	6.54	8.84	10.74	15.71
20	15.90	24.88	6.99	9.24	15.29	21.55	7.33	10.27
30	17.36	28.14	6.23	6.55	16.53	24.78	6.82	7.58
40	18.26	29.49	5.98	5.01	16.67	25.10	7.33	7.33

showed a positive correlation with increasing soil moisture levels for both leaf morph populations. However, at H2 this trend was reversed and increasing soil moisture levels were associated with decreasing R_W values. There was no significant ($P < 0.01$) variation in R_W amongst the moisture levels though it did not vary significantly between the two leaf morph populations. At H2, the R_W values decreased considerably ($P < 0.01$) as compared to values at H1.

The relative N accumulation rates (R_N) in both the leaf morph populations showed a trend similar to that of R_W (Table 7.6).

DISCUSSION

White clover plants have a high water content and thus plant growth is extremely sensitive to moisture stress, resulting in plants becoming dwarf (Johns & Lazenby 1973, Thomas 1984). This view finds support from the findings of Bartels (1966) and Stiles (1966) that the white clover content in mixed swards can be considerably increased by irrigation. The plants in the unwatered treatments failed to survive beyond a 12 day period, but those exposed to 10% soil moisture could grow and nodulate normally. Increased soil moisture contents (up to 30%) brought about a sharp increase in the plant biomass accompanied by an increment in the nodule number per plant of both the leaf morph populations at H1 following which the nodule number dropped at higher soil moisture levels although plant biomass continued to increase. However, at H2 the nodule number per plant increased considerably and maximum

Table 7.6. Analysis of variance of growth parameters of the legume as influenced by harvests, leaf morph populations and different moisture regimes.

Growth parameters	Source of varaition	df	F value	Level of significance
R_W	Harvests	1	881.99	$P < 0.01$
	Morphs	1	0.30	ns
	Moisture %	3	50.40	$P < 0.01$
	Harv X Morphs	1	13.92	$P < 0.05$
	Harv X Moist	3	238.02	$P < 0.01$
	Morph x moist	3	0.01	ns
R_N	Harvets	1	637.31	$P < 0.01$
	Morphs	1	4.99	ns
	Moisture %	3	17.02	$P < 0.05$
	Harv x Morphs	1	21.02	$P < 0.05$
	Harv x Moist	3	168.13	$P < 0.01$
	Morph x moist	3	0.01	ns

ns = not significant

nodulation was observed at 20% soil moisture, but with further increase in soil moisture content, nodulation declined (Fig. 7.1).

Rhizobia are small to medium sized organisms - 0.5-0.9 x 1.2-3.0 μm - and it has been reported that they are unable to move actively over significant distances (Hamdi 1971). Passive transport via soil water to lower depths is rare as the rhizobial cells are usually retained either due to adsorption (Tan _et al_. 1991) or as a result of sieving effect (Bitton _et al_. 1974). As a result, _T. repens_ exposed to higher soil moisture at H2 may find inadequate rhizobial population in the root hair region thereby resulting in low nodule number.

Though the increase in mean nodule weight at H2 as compared to H1 is significant ($P < 0.01$), a reduction in the number of nodules per plant is not compensated for by an increase in the size of the nodules which indicates that extremes of soil moisture are detrimental to both root hair infection and nodule growth. Since stressed conditions imposed due to extremes of soil moisture result in nodules being shed from the roots, it is difficult to determine whether such stress is actually detrimental to nodule growth. At lower soil moisture levels, this fall in nodule number is partially compensated for by the development of juvenile nodules but this compensatory mechanism probably fails at high moisture levels due to the absence of a substantial rhizobial population in the rhizosphere as discussed above. Therefore, the failure to compensate reduced numbers by enhanced growth of surviving nodules results in the nodule mass per plant being proportional to the nodule number (Figs. 7.1a,b).

Studies on Rhizobium have indicated that these bacteria are carried passively in water flowing through saturated soils (Roughley & Worrall 1984) and according to Hamdi (1974) and Madsen & Alexander (1982), percolating water increases the vertical movement of rhizobia through soils. Water flow due to water uptake by plant roots may enhance the movement of bacteria towards root surfaces (Breitenbeck et al. 1988) and the bacteria may also move along or with the roots which may act as a vector in transferring them to greater depths (Bashan et al. 1986; Madsen & Alexander 1982). The development of a small number of nodules per plant at 10% soil moisture, especially at H1, could be attributed to the susceptibility of the clover as well as R. trifolii to relatively drier conditions. T. repens is particularly sensitive to soil moisture as highlighted above. The root nodule bacteria are also severely affected by this stress factor and their susceptibility is known to be proportional to the amount of water retained by cells during drying (Bushby & Marshall 1977) and to the organic matter content of soils. According to Davey et al. (1989), drying of soils, particularly of the uppermost layer of the soil profile, caused nodule number in clover to decrease by 50-90%. Worrall & Roughley (1976) reported that this resulted from a sharp drop in the number of infection threads and nodulation by R. trifolii was consequently inhibited even if the rhizobial population was not severely affected. According to Vincent et al. (1962) and Bromfield et al. (1983), a considerable decrease in the bacterial population due to low soil moisture could also be expected. At low soil moisture, rhizobia survive within water 'lenses' formed within soil pores but movement of the bacteria is severely restricted as such 'lenses' do not form an extensive network of continuous pathways (Griffin & Quail 1968). This view has been supported by Marshall (1971) and Hamdi (1971). Low soil moisture also retards the rate of infection of root hairs (Worrall & Roughley 1976), nodule growth and activity (Issa et al. 1993) and leads to the shedding of pre-formed nodules (Nelson 1983). Such restrictions could explain the reduced nodule numbers at 10% soil moisture.

The above-mentioned findings could help explain the increase in nodule number per plant associated with higher soil moisture levels which would result in enhanced accumulation of rhizobia in the rhizospheric region following passive transport by water (Hamdi 1971). Higher levels of soil moisture, besides triggering off a flush of shoot growth, also makes nutrients such as P, available to the legume which results in significantly ($P < 0.01$) increased plant biomass at successively higher moisture levels (Table 7.1). This would not only enhance growth of belowground root systems thereby increasing possible sites of infection, but also make more photosynthates available to newly formed nodules. Soil moisture is also related to the rate of infection of legume root hairs (Worrall & Roughley 1976). However, at 40% soil moisture, edaphic conditions conditions are exposed to transitory inundation. Excessive soil moisture limits aeration and drastically reduces rhizobial population (Bergersen 1971). Thus in spite of significantly ($P < 0.01$) increased plant biomass, which would have ensured both an enhanced supply of photosynthates and an increase in possible sites of infection, the nodule number drops sharply. This could be attributed to the presumed fall in rhizospheric rhizobial population at this high soil moisture level. According to Graham (1982), excessive soil water results in shedding of existing nodules after only 2 days of imposition of the stress. Recovery from stress may also take considerable time if inundation occurred soon after initial nodulation. Presumably, a percentage of pre-formed nodules were shed leading to reduced numbers. Rapid renodulation under such stress does not occur as it poses a severe stress on carbohydrate reserves of the host.

Prolonged exposure to the above-mentioned soil moisture levels leads to a highly significant ($P < 0.01$) increase in plant biomass (Fig. 7.1a) as well as a sharp rise in the nodule number per plant up to 20% soil moisture in both leaf morph populations (Fig. 7.2a). However, the steep drop in nodule number with further increase in soil moisture could be attributed to the view that moisture levels which prove conducive for maximum nodulation at H1 are detrimental over a prolonged period probably bacause long term exposure leads to excessive accumulation of water thereby limiting soil aeration. Temporary inundation could also lead to pre-formed nodules being shed and the reduction in nodule numbers at H2 as compared to H1 at 40% soil moisture (Fig. 7.2a) lends support to this view.

Comparatively lower values for total N content per plant (Fig. 7.2c) corresponding to low soil moisture could be attributed primarily to poor nodulation exhibited by the legume. Water stress is also known to cause irreversible damage to the symplastic connections between cells of existing nodules, destroying nodule function (Sprent 1976) and their shedding from host plants (Dalton & Zobel 1977). Reduced soil moisture also leads to a sharp decrease in the acetylene reduction assay rates (Sprent 1971, Minchin & Pate 1975) which may ultimately come to a halt (Davey & Simpson 1989). This is probably brought about due to reduced respiration rates resulting in lowered N_2 fixing efficiency of stressed nodules. This view finds support from the studies of Engin & Sprent (1973) and DeJong & Phillips (1982). On the contrary, reduced N accumulation at high soil moisture levels could be attributed primarily to the steep drop in nodule number and nodule mass per plant leading to reduced N_2 fixing tissue.

There was no significant difference between the marked and unmarked leaf morph populations with respect to any of the parameters studied except for nodule number and root biomass, both of which were greater in the marked plants. Since both the leaf morph populations were exposed to similar soil moisture levels and watering frequencies, soluble NO_3 ions would have been equally leached leading to marked plants becoming comparatively N-starved owing to their higher N requirement. These plants therefore develop a larger number of N_2 fixing root nodules as a means of overcoming this crisis.

Results of the present study indicate that nodulation is best achieved at intermediate soil moisture levels which appear to be between 10-20% on dry weight basis. This amount of soil moisture appears to prevent prolonged desiccation, provides conducive conditions for growth of both the legume and the bacteria and, at the same time, allows adequate aeration by preventing waterlogged conditions to be manifested. Though this range of soil moisture is recorded at the swards during the major part of the year, the dry winter spells lowered soil moisture to levels well below the above-mentioned range. Drying of soils coupled with low temperatures during these months could act as a major stress. However, rhizobia are known to withstand such stressed spells by surviving within water 'lenses' (Issa et al. 1993) and can multiply and re-establish their population the following spring. Clover nodules which manage to overwinter are also known to respond effectively to re-watering, due to their indeterminate nature, and can thereby continue to grow and function in the following spring with the return of favourable conditions.